The Pharmacological Assay of Digitalis by Different Methods

BY

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THE PHARMACOLOGICAL ASSAY OF DIGITALIS BY DIFFERENT METHODS.

By J. W. Trevan and Ellen Boock, J. H. Burn and J. H. Gaddum.

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In 1924 an investigation of the relative potency of three samples of digitalis leaves was organised on behalf of the Health Section of the League of Nations. The samples were distributed to pharmacologists in different countries, and their reports are summarised in a paper by Knaffl-Lenz.[1] The results obtained by different workers using the cat method agreed on the whole better with one another than did those in which the frog method was used, but there was some disagreement between the results of the cat method and those of the frog method.

In 1926 comparisons of two tinctures of strophanthus[2] and of two tinctures of squill[3] were carried out by three of us (J.W.T., E.B., and J.H.B.), using different methods. Each method assayed the tinctures of strophanthus in terms of ouabain and the tinctures of squill in terms of scillaren. The results by the cat and the frog method for the strophanthus tinctures agreed with an approach to the

precision of a chemical estimation, and, with one curious exception, the results for the squill tinctures agreed well too. The peculiar feature of these comparisons was the application of a method of using frogs which has been devised by two of us (J.W.T. and E.B.), which enables the variation between different animals to be treated systematically. It was of interest to determine whether the examination of digitalis leaves in terms of the international standard powder would show better agreement between the results by the cat method and the frog method when this device for carrying out the frog test was applied. Accordingly, two samples of digitalis leaf, described hereafter as D and U, have been compared with the international standard powder. The work was done independently in three laboratories, and the results were not communicated from one to the other until all were complete.

Report by J. W. Trevan and Ellen M. Boock
(*From the Wellcome Physiological Research Laboratories*).

From each of the three powders, tinctures were prepared by grinding 9 gm. of powder with 90 c.c. of 70 per cent. alcohol in a ball mill for twenty-four hours. The liquid was then filtered, and the filtrate used for the biological test.

1. *Estimation of Strength by Hypodermic Injection into Frogs.* (*R. temporaria.*)

For the purpose of this estimation, the ordinary procedure of injecting a number of pairs of frogs with a series of diminishing doses has been discarded. Instead, use has been made of a standard curve, relating percentage mortality to dose, which was determined by us for one tincture of digitalis by the use of several hundred frogs. This curve is reproduced in figure 1 (see page 10). It shows that there is a wide variation, nearly 500 per cent., between a dose of a tincture large enough to kill some frogs and that large enough to kill all. The abscissæ are arbitrary, the dose which kills 50 per cent. of frogs having been given the value 4.

The test is carried out in the following way. In order to compare two tinctures, a large group of frogs, for example 24, or 30 or 40, is taken for each tincture. A dose, reckoned in c.c. tincture per 100 gm. body weight of frog, is then chosen for each tincture, and this dose is given to each of the frogs in the group. The volume injected into each frog is always 0·5 c.c. A certain proportion of the frogs die.

and this proportion is expressed as a percentage. From the curve the abscissa corresponding to this percentage mortality is then observed. This abscissa expresses the activity present in the dose of tincture injected, and the relative potency of the two tinctures is determined from the relation between abscissæ obtained in this way. An example will serve to explain the method. In experiment B, Table 1, a dose of 0·4 c.c. tincture S per 100 gm. body weight of frog was injected into 12 frogs. Next morning, 7 frogs were dead, so that the percentage mortality was 58·3. At the same time a dose of 1·0 c.c. tincture D was given to 12 frogs, with the result that 11 died. The percentage mortality was 92. From the curve, the abscissa corresponding to 58·3 per cent. is 4·15, and that corresponding to 92 per cent. is 5·05. Hence $\frac{0{\cdot}4 \text{ c.c. S}}{1{\cdot}0 \text{ c.c. D}} = \frac{4{\cdot}15}{5{\cdot}05}$ and $\frac{\text{D}}{\text{S}} = 0{\cdot}488$, or D = 48·8 per cent. of S.

The total number of observations made in this way is given in Table 1, the table showing the number of frogs which died (*a*) three hours after the injection, (*b*) overnight.

TABLE 1.

Tincture.	Dose c.c. per 100 gm. frog.	Result in three hours.	Overnight.
Experiment A.			
S	0·6	10/15 = 66·6%	15/15 = 100%
	0·5	9/15 = 60%	14/15 = 93·3%
U	0·4	12/15 = 80%	14/15 = 93·3%
	0·3	8/15 = 53·3%	14/15 = 93·3%
D	0·7	3/15 = 20%	4/15 = 26·6%
	0·6	3/15 = 20%	4/15 = 26·6%
Experiment B.			
S	0·4	5/12 = 41·6%	7/12 = 58·3%
	0·3	0/12 = 0%	0/12 = 0%
U	0·2	1/10 = 10%	2/10 = 20%
	0·15	1/12 = 8·3%	1/12 = 8·3%
D	1·0	9/12 = 75%	11/12 = 92%
	0·8	7/12 = 58·3%	9/12 = 75%
Experiment C.			
S	0·4	5/40 = 12·5%	9/40 = 22·5%
U	0·22	7/40 = 17·5%	9/40 = 22·5%
D	0·85	29/40 = 72·5%	31/40 = 77·5%

The activity of tinctures U and D determined in terms of tincture S is given for each experiment in Table 2.

TABLE 2.

Tinctures.	Doses in c.c. per 100 gm. frog.	Experiment.	Three hours ratio.	Overnight ratio.
U and S	0·6 S and 0·4 U	A	160·5%	?
	0·5 S and 0·4 U		137	125
	0·6 S and 0·3 U		186	?
	0·5 S and 0·3 U		159	166·7
	0·4 S and 0·2 U	B	138·8	156
	0·3 S and 0·2 U		?	?
	0·4 S and 0·15 U		169	169
	0·3 S and 0·15 U		?	?
	0·4 S and 0·22 U	C	195	181·8
D and S	0·6 S and 0·7 D	A	63·7	?
	0·5 S and 0·7 D		53·2	47·6
	0·6 S and 0·6 D		74·4	?
	0·5 S and 0·6 D		62	53·5
	0·4 S and 1·0 D	B	45·9	48·8
	0·3 S and 1·0 D		?	?
	0·4 S and 0·8 D		55·3	54·3
	0·3 S and 0·8 D		?	?
	0·4 S and 0·85 D	C	73	65·2

(N.B.—" ? " indicates that the results could not be used because either none of the frogs, or all of them, died.)

In this way a series of figures for the value of both D and U in terms of S was obtained. To obtain the final figure for the two tinctures it was clearly not sufficient to take the mean of the different results, for each of these depended for its accuracy on the number of animals employed in obtaining it. A final mean was obtained by multiplying each result by the number of animals used to obtain it, taking the sum of the products and dividing the sum by the total number of animals used. The figures obtained for D and for U appear in Table 3 (see page 10).

The standard deviation of these results can be obtained approximately from the formula (Trevan[4]):

$$D^2 = \frac{180{,}000 \text{ p.q. } K^2}{N}$$

where " D " is three times the standard deviation, " p " and " q " the average probabilities of mortality and survival at the doses used (which on the average can be taken as 0·5),

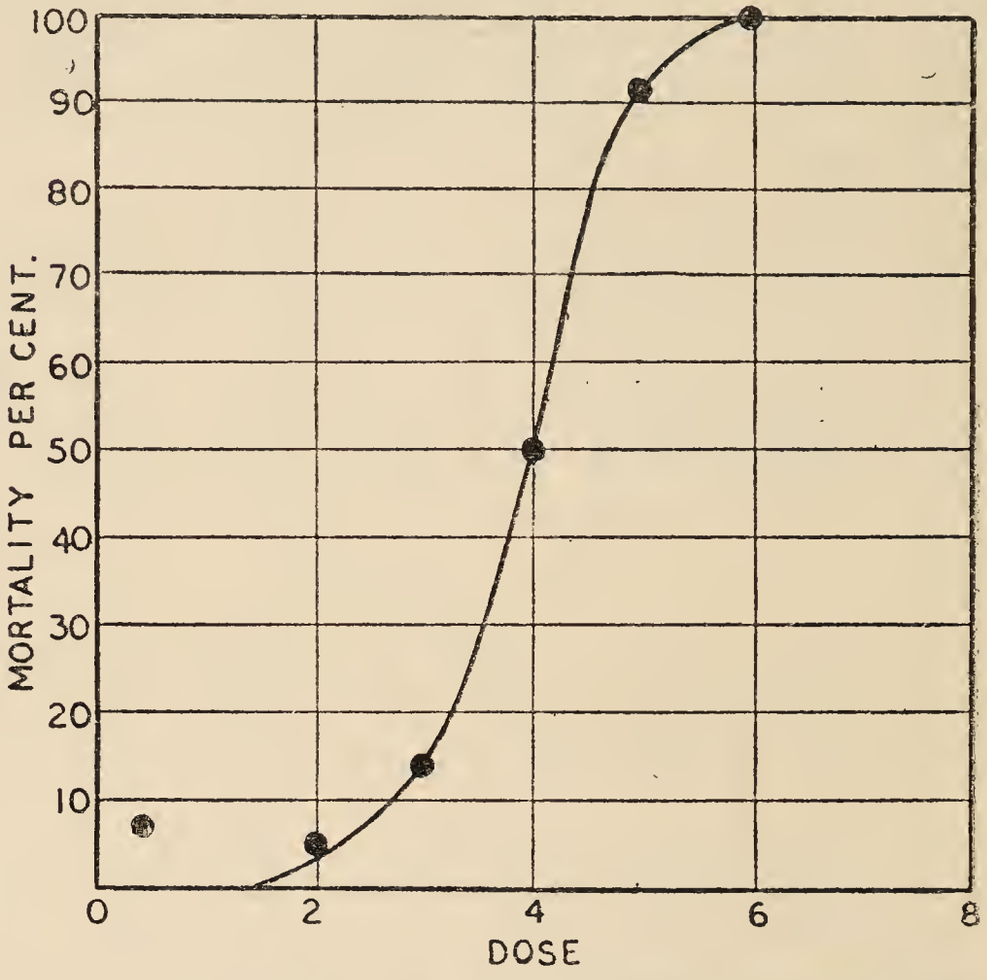

FIG. 1.—Curve Relating the Dose of Digitalis to the Percentage Mortality of Frogs. The Dose producing 50 per cent. Mortality is given the Arbitrary Value 4.

K the percentage increment of dose for 1 per cent. increment of mortality for the standard curve (= 0·603), N the number of animals injected with each tincture (= 79) (counting only those animals from the groups in which only some of the animals died, and neglecting those in which 0 or 100 per cent. mortality was obtained. These have also been omitted from the calculations in the tables). The standard deviation

TABLE 3.

U in terms of S. Three hours 175·2%	Overnight 167·2%
D in terms of S. Three hours 65·4%	Overnight 57·9%

so calculated is about 5 per cent., so that the difference between the observed figures for the means of the different batches is significant.

The ratio of the three batches can also be estimated without reference to any standard curve by the method first used by Krogh for insulin, in which the mortality is plotted against the logarithm of the dose, and parallel straight lines are drawn through the three sets of points obtained. The horizontal differences between the three lines then give the

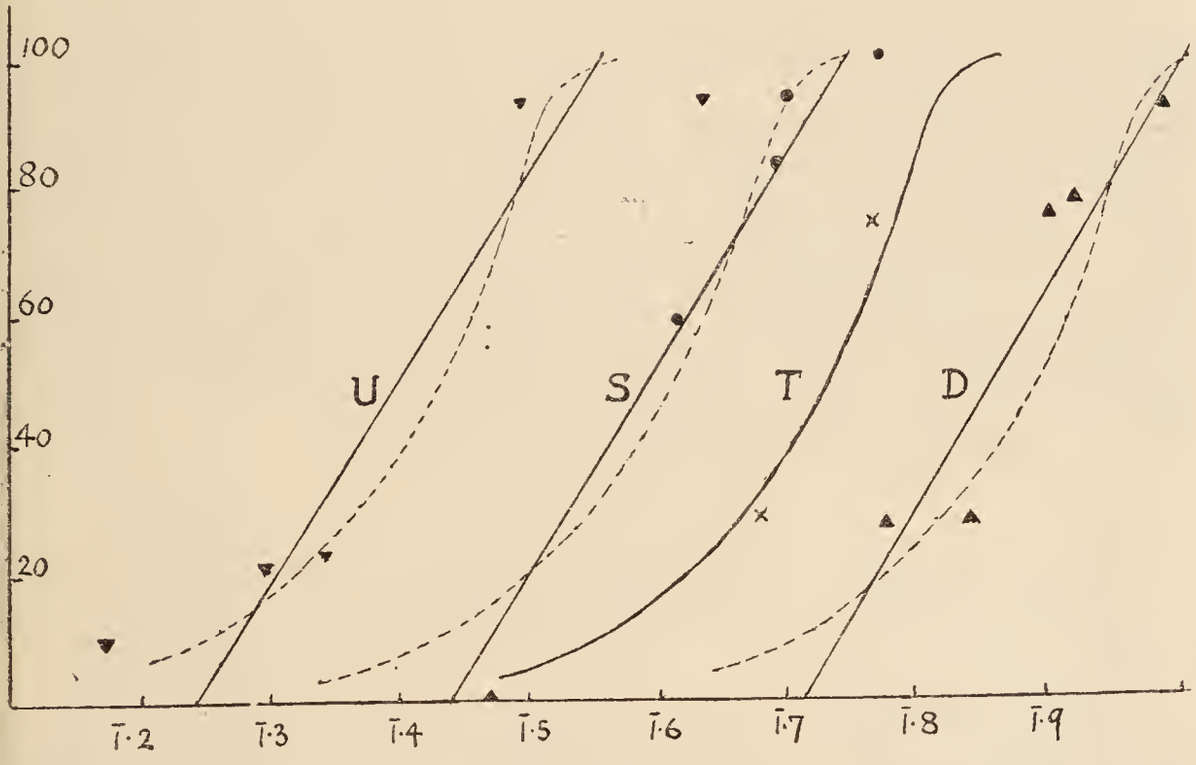

FIG. 2.—Curves showing a Comparison of the Tinctures Prepared from the Leaves U, D and standard (S) and of a Solution of Ouabain (U.S.P.) containing 0·083 mgm. per c.c. The ordinates are the percentage mortality of the frogs, and the abscissæ are the logarithms of the dose. The percentage mortalities actually obtained are given by the points ● ——— ● for S; ▼ ——— ▼ for U; ▲ ——— ▲ for D; × ——— × for Ouabain. The curves drawn are similar to that in Figure 1, but are altered in shape by the logarithmic scale.

logarithm of the ratio of the activities. (See figure 2.) The horizontal distance between the line drawn for S and U is 0·2, the antilogarithm of which is 1·585, and the distance between S and D is 0·254, the antilogarithm of which is 1·79, so that the activity of U in terms of S is 158·5 per cent. and D in terms of S is $\frac{100}{1\cdot79} = 55\cdot8$ per cent.

At present we are using the United States sample of ouabain as a routine standard, 0·083 mgm. of which should be equivalent to 1 c.c. of tincture of digitalis of the United States Pharmacopœia. The curve for ouabain taken from our routine figures during the time of the present experiments is plotted at T in figure 2. The dotted curves are parallel

to this curve and show the degree of agreement between the observed distribution of the mortalities and the ouabain curve. The distance between this curve and the curve drawn through S indicates that 1·0 c.c. of the tincture of S is equivalent to slightly more than 0·1 mgm. of ouabain, when the comparison is carried out on frogs.

2. *Estimation of Strength from Effect on Isolated Auricles of Rabbit.*

The tinctures have also been compared by means of observations on the isolated auricles of the rabbit. We have found that when these are suspended in a Ringer's solution buffered with boric acid and borate so that the *p*H is about 7·6, they regularly show a reversible increase of amplitude when a digitalis preparation is added to the bath. The increase is proportional to the dose added, and hence may be used as a means of comparing the strengths of two preparations.

The formula for the Ringer's solution is

NaCl	9·0	gm.
KCl	0·42	„
$CaCl_2$ (reckoned as anhydrous salt) ...	0·24	„
Dextrose	1·0	„
Boric Acid (M/5)	17·0	c.c.
Borax (M/20)	3·0	„
Distilled water ... ... to	1,000	„

The digitalis tincture is used without dilution, after first removing the bulk of the alcohol on the water bath, and then adding saline to bring back to the original volume. A dose of 0·2 c.c. may be added to a bath of 25 c.c.

In performing the test the digitalis is allowed to act on the auricles for one minute, at which time a record of the amplitude of the beat is obtained ; the drum is then stopped and turned on again after four minutes more to obtain a fresh record of the greater amplitude. The solution in the bath is then removed, and replaced with fresh Ringer's solution. After a rest of twenty minutes, in which the amplitude has diminished to its original size, a further dose of digitalis is added. An effort is made to find doses of two preparations which produce the same increase of amplitude.

By this method, tincture U was found to be 166 per cent. and tincture D to be 75 per cent. of tincture S.

The difference in the result by this method for D and that

obtained by the subcutaneous frog method is, we think, probably significant. (We have met with this type of discrepancy before, particularly with a sample of the mixed glucosides from digitalis leaf. This sample in a 1 per cent. solution was 2·3 times the value of a standard tincture when tested on the auricle, but was equal to standard by the frog method.) We therefore attempted to discover the dose for the frog's heart, when applied directly.

3. *Estimation by Intravenous Perfusion into Frogs.*

The diluted tincture was injected slowly into the caudal end of the anterior abdominal vein of the pithed frog, by means of a micrometer syringe[6] with a glass cannula connected by a ground glass joint. The frogs were immersed in a bath of saline at 27°C., and 0·002 c.c. of the diluted tincture was injected every ten seconds, until the heart stopped in systole. The results are given in Table 4, together with the standard deviations worked out by the mean squares method. The result for D certainly differs by a significant amount from the results obtained by the subcutaneous method.

TABLE 4.

No. of frogs.		c.c. of original tincture per 100 gram frog.	Standard deviation.
S	7	0·217	0·006
U	7	0·129	0·0088
D	10	0·2418	0·014

U is therefore 168 per cent. of S, and D 89·8 per cent.

The collected results by different methods are shown in Table 5.

TABLE 5.—*Collected results by different methods.*

Method.	U in terms of S.	D in terms of S.
Subcutaneous Frog:		
Calculated method	167·2%	57·9%
Graphic method	158·5	55·8
Isolated auricle of rabbit . . .	166·6	75
Intravenous perfusion of frog . .	168	89·8

Report by J. H. Gaddum

(*From the National Institute for Medical Research, London, N.W.3*).

A. *Comparison of Samples S, U and D using the Isolated Auricles of the Rabbit.*

1. *Method of preparing the extracts.*—The leaves were continuously extracted with absolute alcohol by placing 2 gm. in a Soxhlet thimble and suspending the thimble beneath a reflux condenser fitted in a flask. The alcohol, of which 75 c.c. were used, was allowed to boil for three hours. The condensed alcohol vapour dripped back through the thimble into the flask, extracting the leaves on its way. The extract was taken down to about 5 c.c. and made up to 50 c.c. with the Ringer's solution used for the isolated auricles. (See report of Trevan and Boock.) On adding the Ringer's solution a precipitate was formed which, before the liquid was added to the bath, was uniformly distributed by shaking.

2. *Performance of the test.*—The test was carried out as described in the report of Trevan and Boock, and the results obtained were :—

Extract of leaf U was 157 per cent. of Extract S.
" " " D " 45 " " " " S.

A mixture of equal volumes of U and D was 100 per cent. of Extract S.

B. *Comparison of the Samples by the Guinea-Pig Method of Knaffl-Lenz.*

1. *Method of preparing the extracts.*—A known weight of leaf, between 1-2 gm., was extracted with 80 c.c. of water in a porcelain dish for 15 minutes at 80°C. The solid particles were separated by centrifugation and washed in succession with 10 c.c. of water and 10 c.c. of 9 per cent. saline. The washings were added to the extract to make a final volume of 100 c.c.

2. *Method of performing the test.*—I am much indebted to Dr. Knaffl-Lenz for advice and help in carrying out the comparisons. The technique he has described[1] is essentially similar to that used by de Lind van Wijngaarden[5] in the cat method. Guinea pigs weighing 500-900 gm. are used. The digitalis extract is infused into the jugular vein at a slow, steady rate, while the heart is palpated through the chest wall. The final arrest of the heart is determined by inspection through an opening in the chest wall.

Throughout the experiment the lungs are artificially inflated with air blown over ether. Table 6 gives the figures for the lethal dose of each leaf expressed in mgm. of leaf per 100 gm. weight of guinea pig.

TABLE 6.

Tincture U.	Tincture D.	Tincture S.
10·7	41·5	14·8
10·4	33·3	14·3
11·6	26·7	13·1
8·45	39·4	14·1
9·5	40·2	17·8
9·0		16·0
8·6		13·4
11·0		
10·8		
Mean 10·0 ± 0·36	36·2 ± 2·5	14·8 ± 0·6

The figures following the ± sign are the standard deviations of the mean, determined from the formula

$$\sqrt{\frac{\Sigma d^2}{n}} \times \frac{1}{\sqrt{n}}$$

In examining the extract from leaf D, it was found that a disproportionately large volume had to be injected before arrest of the heart occurred. Accordingly a 3·5 per cent. extract was prepared, with which the first three determinations in Table 1 were made. These showed a progressive increase in potency which it seemed might be due to some active principle settling down to the bottom of the burette used for infusing the drug. The last two determinations were obtained with a 2·5 per cent. extract.

The relation of the average figures in Table 1 is

U = 148 per cent. of the strength of S.
D = 41 „ „ „ „ „ „ S.

Report by J. H. Burn

(From the Pharmacological Laboratory, Pharmaceutical Society of Great Britain).

Comparison of three Samples of Digitalis Leaf, S, U and D.

1. *Method of preparing solutions for use.*—Tinctures were prepared from each sample of leaf according to the

directions for preparing the official tincture of the British Pharmacopœia, 1914.

2. *Determinations by the Cat Method.*—The technique employed was that described by de Lind van Wijngaarden (*loc. cit.*) with the addition that the blood pressure in the carotid artery of each cat was recorded. The end point of the determination was the final fall of the blood pressure to zero, as shown on the kymograph. For infusion into the cat, each tincture was diluted twenty times with 0·9 per cent. saline. The figures in Table 7 are the individual lethal doses for different cats in c.c. of the diluted tincture per kgm. of body weight.

TABLE 7.

Tincture U.	Tincture D.	Tincture S.
7·89	17·75	12·35
9·35	18·8	14·5
10·75	18·9	14·8
11·27	20·51	15·4
13·94	21·54	16·9
13·94	26·9	17·9
14·14	29·18	19·37
Average 11·61 ± 0·86	21·94 ± 1·54	15·96 ± 0·85

The figures following the ± sign are the standard deviations of the corresponding averages.

From the averages of the results for each tincture, it was calculated that:

Tincture U is 137 per cent. of Tincture S.
„ D „ 73 „ „ „ „ S.

3. *Determinations by the Guinea-Pig Method.*—The technique employed was that described by Dr. Knaffl-Lenz, who was good enough to demonstrate the method to me. For infusion into the guinea pig, a known volume of each tincture was placed in an evaporating dish on a boiling water bath until the volume was reduced to about one-half. The residue, in which there was no precipitate, was then diluted to 8 times the original volume in the case of tinctures U and S, and to 4 times in the case of tincture D. The lethal doses were then determined for a series of guinea pigs. The results are given in Table 8, expressed in terms of mgm. leaf per 100 gm. weight of guinea pig.

TABLE 8.

Tincture U.	Tincture D.	Tincture S.
7·18	22·7	8·6
9·65	29·4	10·3
10·04	30·0	10·5
10·6	31·5	10·6
10·6	37·5	11·0
12·24	38·75	13·08
		17·2
		18·5
		20·18
Average 10·05 ± 0·6	31·64 ± 2·19	13·33 ± 1·29

From the averages of the results for each leaf, it was calculated that:

Leaf U is 132 per cent. of leaf S.
„ D „ 42 „ „ „ „ S.

Discussion.

A summary of the results of the three sets of observers is given in Table 9.

TABLE 9.

Workers.	Method of extracting leaf.	Method of Assay.	Result as percentage of S.	
			U.	D.
Trevan and Boock	Cold alcohol	Frog, 12 hour	158·5	55·8
„	„	„ intravenous	168	89·8
„	„	Rabbit, isolated auricle	166·6	75
Gaddum	Soxhlet (hot alcohol)	„ „ „	157	45
„	Hot water infusion	Guinea-pig	148	41
Burn	Tincture	Cat	137·5	73
„	„	Guinea-pig	132	42

These figures for the different methods were disappointing, for the lack of agreement stands in sharp contrast to the close agreement previously obtained for the relation of two tinctures of strophanthus in terms of ouabain, and for the relation of two tinctures of squill in terms of scillaren.

The differences between the figures for tincture U are, however, less than may appear at first sight ; thus the extreme variation from the mean value of U is not more than 15 per cent., so that the differences in the results may not really indicate that one method would always give a higher result than another. The differences for D, however, are certainly significant, and require consideration. It must be supposed that they are a reflection of the existence in digitalis of different toxic principles which act in a way which differs quantitatively according to the animal used and to the method of administration. The results with D make it clear that, in order to be sure of standardising correctly *every* sample of digitalis, it is unsatisfactory to leave to each worker the choice of the method he shall use. For one who used the intravenous frog method would maintain with reason that this leaf complied with the potency requirement of the Geneva Conference of 1925, having 89 per cent. of the strength of the standard, while another, using the guinea-pig method, would decisively reject it. Since the results with the strong leaf, U, do not show these divergences at any rate to the same extent, it might be supposed that they are a peculiarity of samples of leaf of less than average toxicity. But the experience of one of us with another tincture makes it highly probable that this is not so. This sample by the cat method had 122 per cent. of the activity of the standard, but by the frog method it had as much as 175 per cent. Evidently divergent results by different methods may result from the examination of strong as well as of weak samples.

The question of choosing the right method whereby the most important therapeutic principles are estimated can only be determined by clinical evidence which for the present is not available. It would be of considerable help, however, to determine how often the divergences between the results by different methods are great and how often they are within the limits of the error of any one method as usually applied. It may well be that for the majority of samples these divergences are small, and if this is so the importance of defining the method to be used diminishes.

The International Standard Powder.—Evidence is now available to show how far the international standard powder is representative of an average sample of digitalis in Great Britain. Since the beginning of 1926, samples of digitalis,

chiefly in the form of tincture, have been accepted for standardisation from different manufacturers by the Pharmaceutical Society, and these have been compared with the international standard by the cat method. A few results were obtained by the guinea-pig method also. The percentage strength of these samples in terms of the international standard is shown in figure 3. Out of a total of 43 samples, 32 fell within the limits laid down by the Geneva Conference, while 2 were too weak and 9 were too strong. The evidence supports the view that the international standard is one which is readily attainable by manufacturers in this country.

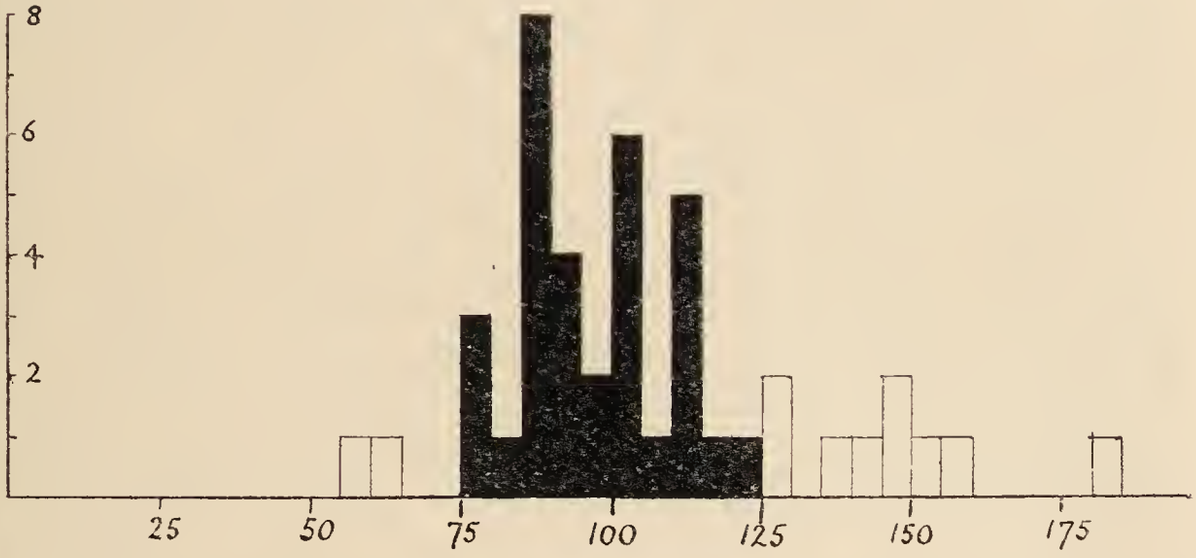

FIG. 3.—Showing Distribution of Samples of Digitalis Leaf and Tincture received by the Pharmaceutical Society in relation to the International Standard. Ordinates are the number of samples, abscissæ the percentage of standard strength. All samples in the black area passed the test. (The values for samples which did not pass the test were all obtained by the cat method.)

Further, the substitution of the international standard for the present ouabain standard will make no appreciable difference in the United States. In the Pharmaceutical Society's laboratory the lethal dose of tincture prepared from the international standard by the cat method was found to be 0·76 c.c. per kgm., and that of the official U.S.P. ouabain to be 0·06115 mgm. per kgm. Hence 1 c.c. of the tincture from the international powder was equivalent to 0·0804 mgm. ouabain. But the present U.S.P. requirement is that 1 c.c. of a tincture shall be equivalent to 0·083 mgm. ouabain, so that the international tincture is for practical purposes the same as a tincture fulfilling the U.S.P. definition. It has previously been pointed out, however, that on the frog the

equivalent of the international standard is 0·118 mgm. of ouabain per c.c. This is clear evidence of the unsuitability of ouabain as a standard for digitalis.

Samples which do not Satisfy the Geneva Recommendation.—While, then, there is evidence that the international standard is likely to be readily attained by manufacturers in different countries, on the other hand we think it doubtful whether the form of recommendation concerning leaves which are too weak or too strong has been well chosen. The sentence reads "Only such leaves shall be passed for issue as differ from the standard preparation by not more than 25 per cent."

The question of leaves which are too weak may be considered first. Provided that in all countries manufacturers find the standard to be one which is readily attained, we agree that leaves less than 75 per cent. of the strength of the standard should never be passed for issue. Certainly we think that in incorporating standardised digitalis into the British Pharmacopœia, leaf weaker than 75 per cent. of standard should under no circumstances be allowed. But subsequent investigation may reveal that the average leaf of some countries is less than the standard in strength ; it is certainly not inconceivable that leaf grown in tropical or sub-tropical climates may have a different average potency from that grown in temperate zones. The present recommendation leaves no room for this contingency, and where it occurs the recommendation cannot be made operative.

The difficulties of the recommendation become greater, however, when applied to leaf which is more than 25 per cent. stronger than the standard. Of the samples examined by the Pharmaceutical Society, about one in five were in this class. The recommendation prohibits the use of this leaf unless it is mixed with a leaf of low potency so that the strength is within the 25 per cent. limit of variation from the standard. The recommendation implies that digitalis leaf cannot be classified into good and bad leaf, but only into strong and weak. The evidence does not permit us at present to deny this, but we think that the purpose of standardisation can equally well be served by recommendations which make no implication whatever. Whether or not it is true that a strong leaf is qualitatively as well as quantitatively superior to a weak one, a view to which we incline, it is at least clear that the necessity for degrading strong leaf with weak is a deterrent to the enterprising grower who seeks, by improving his manuring and methods of cropping, to produce

a preparation of the highest glucosidal content. This is a result of the recommendation which we think most undesirable.

It may not be realised, perhaps, that the exclusion of leaf which differs from the standard by more than 25 per cent., is a departure in the case of digitalis from the principle followed in the cases of pituitary extract and insulin. For these last two substances the Conference was content to approve the adoption of a particular sample as standard, and to indicate how other samples should be measured in terms of that standard. No recommendation was made that the strength of samples sold in all countries should have any precise value. It seems to us that the position of digitalis would be simplified if the recommendations concerning it conformed to those for the other substances. It is sufficient to adopt a standard and to lay down that the strength of all preparations be expressed in terms of the standard. It would then be permissible for any country, in dealing for example, with a tincture prepared from an unusually strong leaf, to require it to be diluted to equivalence with standard tincture; or, in dealing with leaf issued in tablet form or in capsules, to require that the amount of leaf in any one tablet or capsule shall be a weight which contains activity equivalent to that in a given weight of standard leaf. In this way a proper advantage is given to the grower of a strong leaf over the grower of a moderate or weak one.

A good case can be made for treating digitalis in exactly the same way as insulin and pituitary extract have been treated, by adopting a unit of digitalis to represent the activity contained in a given weight of the standard powder. This course has the merit of extreme simplicity which makes it attractive. It has, however, certain disadvantages. Before the introduction of the unit of pituitary extract, the general practitioner was entirely unable to understand the relation between the strengths of preparations made by different manufacturers. His bewilderment has now disappeared and he regards the unit as a valuable conception. The present position of digitalis, however, presents him with no similar difficulties, and it is probable that he would regard a unit as an unnecessary complication of a position already simple. It would seem desirable in the case of a long-established remedy, in which the ordinary biological variation, unlike the obvious divergences which existed in pituitary extract, is relatively small, that the pharmacologist should effect its standardisation by regulations which concern only himself and the

pharmacist, and of which the general practitioner would remain entirely unaware.

The balance of considerations seems to us, therefore, to be against the introduction of a unit, but we feel, as we have already said, that the position would be materially simplified if the Conference were content to leave details of the mechanism for securing uniformity to each country, demanding only the acceptance of the international standard and the expression of potency in terms of that standard.

(N.B.—No reference has been made in the course of the paper to the sources from which the powders examined were obtained. The composition of the standard powder has been described by de Lind van Wijngaarden.[7] Powder U came from Mr. Upsher Smith, being generously supplied free of charge for the purpose of providing material for a British standard. It was a bright green powder which had been dried at 55°—60°C. from a 1925 crop. Powder D came from Holland, and was a brown powder of 1925 crop.)

Summary.

1. The results of assaying two samples of digitalis leaf, the one strong and the other weak, in terms of the international standard digitalis powder, using different methods, are described.

2. The agreement obtained between the assay by different methods of the strong sample was fairly good, the discrepancies being not greater than those obtained when different workers use the same method.

3. There was serious disagreement between the different results for the weak sample, though all methods agreed in showing that the sample was weak.

4. Evidence is given showing that the international standard powder is a practicable standard for digitalis leaf and tincture in Great Britain.

5. It is recommended that the prohibition by the Geneva Conference of the use of leaf which differs from the standard by more than 25 per cent. be withdrawn, and that having adopted a standard, in terms of which the strength of all leaf should be expressed, the International Conference should be content, as in the case of insulin and pituitary extract, to leave each country to arrange the mechanism of standardisation for itself.

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